Supplemental Material

Methods

All experimental studies using human samples comply with the Declaration of Helsinki and were approved by the local ethics committee (*Comité de Protection des Personnes* [CPP] EST-II n°: 18.06.06). All patients gave informed consent before the study.

Isolation, and culture of primary Human pulmonary endothelial cells (ECs): Human pulmonary ECs were isolated and cultured as previously described [16,17]. The isolated ECs were strongly positive for acetylated low-density lipoprotein coupled to Alexa 488 (Alexa488-Ac-LDL), von Willebrand factor (vWF), CD31, and for Ulex europaeus agglutinin-1 (UEA-1) and negative for alpha-smooth muscle actin (α-SMA). Cells were routinely tested for mycoplasma and used at early passages ≤ 5.

Immunofluorescent staining and microscopy: Immunofluorescent staining for NF2 (1:100; sc-55575 from Santa Cruz), α-SMA (1:200; sc-32251 from Santa Cruz), and vWF (1:200; A0082 from DAKO) were performed in lung paraffin sections as previously described [11,12]. Briefly, lung sections (5µm of thickness) were deparaffinized and incubated with the antigen retrieval buffer. Then, sections were saturated with blocking buffer and incubated overnight with specific antibodies, followed by addition of the corresponding secondary fluorescent-labelled antibodies (Thermo Fisher Scientific, Saint-Aubin, France). Nuclei were labelled using DAPI (Thermo Fisher Scientific). Mounting was performed using ProLong Gold antifade reagent (Thermo Fisher Scientific). All images were taken using a LSM700 confocal microscope (Zeiss, Marly-le-Roi, France).

Western Immunoblot: Cells were homogenized and sonicated in PBS containing protease and phosphatase inhibitors (Sigma-Aldrich). Fifty µg of protein extract were used to detect NF2 (sc-55575; 1:200) by SDS-PAGE as previously described [11,12]. Statistical significance was tested

using the nonparametric Mann-Whitney U test. Significant difference was assumed at a p value of < 0.05. Analyses were performed using GraphPad Prism v5.0, La Jolla, CA.

Refrences

11. Savale L, Akagi S, Tu L, et al. Serum and Pulmonary Uric Acid in Pulmonary Arterial Hypertension. *Eur Respir J.* 2021.
12. Tamura Y, Phan C, Tu L, et al. Ectopic upregulation of membrane-bound IL6R drives vascular remodeling in pulmonary arterial hypertension. *The Journal of clinical investigation.* 2018;128(5):1956-1970.